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16. ABSTRACT

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JOURNAL ARTICLE

Prenatal Effects of Herbicides: Evaluation by the Prenatal Development Index[1]

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Abstract. The herbicides 2,4-D and 2,4,5-T and many of the esters of these compounds produced cleft palates in CD-1 mice. Silvex and Agent Orange also produced cleft palates. Depending on the dose and means of administration variable responses relating to the production of cleft palates, effect on fetal weight, and effect on fetal mortality were obtained. In order to view these data comprehensibly, the Prenatal Development Index was determined. This index was computed from the incidence of malformed fetuses, fetal mortality and fetal body weight. This made it possible to evaluate data from some experiments with feto-toxic doses of compounds in which the high incidence of fetal mortality and consequently low incidence of viable fetuses obscured the response.

The Prenatal Development Index is a concept that permits the evaluation of combined teratogenic and toxiocologic data. In laboratory studies, after administration of compounds to pregnant animals and examination of the fetuses, the question arises as to whether or not a teratogenic, fetotoxic or fetocidal effect was produced. In some experiments only one or two of the effects were seen. In other experiments all three effects were seen, thus making it difficult to label the action of the compound as teratogenic or fetotoxic. The need to categorize a compound as teratogenic or fetoxic seemed less desirable than the need to evaluate the development of the fetus with an overall perspective. Thus, the Prenatal Development Index was derived to utilize teratogenic, toxicologic and fetocidal data and reduce them to a single expression that could aid in the evaluation of the effects of a compound on fetal development.

In order to present this concept, data were obtained on the fetal effects of a number of herbicides of the phenoxyacetic acid class and related compounds. The herbicide 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) was shown to be teratogenic in mice (Courtney and Moore 1971). Subsequently, a number of studies have used 2,4,5-T as a prototype to explore facets of teratology such as dose-response relationships, interactions, species differences and fetal distribution

[1] Presented in Part at the 15th Annual Meeting of the Teratology Society, May 11–14, 1975, Pocono Manor, Pennsylvania.

[2] The mention of company or product names is not to be considered as endorsement or recommendation for use by the Environmental Protection Agency.

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(Collins and Williams 1971, Binns and Balls 1971, Emerson *et al.* 1971, Khera and McKinley 1971 and 1972, Hall 1972, Lindquist and Ullberg 1971, Roll 1971, Sparchu 1971, Thompson 1971, Neubert and Dillman 1972, and Bage *et al.* 1973). This herbicide has two major attributes for these types of studies: 1) no obvious maternal toxicity at teratogenic dose levels, and 2) the teratogenic effect is discreet in that cleft palates are produced almost exclusively. In addition to these attributes, 2,4,5-T has many structural analogs both herbicidal and non-herbicidal to permit a structure-activity study that would enable a simple comparison between compounds.

Methods

Pregnant CD-1 mice were obtained from Charles River Laboratories, Inc. Compounds were administered by oral gavage or subcutaneous injection as indicated in the tables. All control mice were studied concurrently. All animals were sacrificed on day 18 of gestation; day 1 was marked by detection of a vaginal plug. Upon sacrifice the fetuses were examined and stored in Bouin's solution until they could be examined in more detail.

The compounds were procured from the various companies as indicated in Table 1. For the most part, compounds were analytical grade. The Dow Chemical Co. generously supplied most of the compounds in the 2,4-D and 2,4,5-T series. The compounds in the 2,4,5-T series were supplied with an analysis specifically for the content of 2, 3, 7, 8 tetrachlorodibezo-p-dioxin (TCDD) as indicated in the table.

The following conventions were observed in compiling the data. If a fetus was either dead or resorbed, it was regarded as a dead fetus. Only live fetuses were examined for physical abnormalities. A fetus was classified abnormal if it was alive and had at least one type of anomaly (regardless of type). To calculate the maternal weight gain, the difference in the maternal weight from the beginning to the end of the experiment was determined after subtracting the terminal gravid uterus weight. The maternal liver to body weight ratio was determined at the time of sacrifice using the maternal weight minus the gravid uterus weight. For statistical analysis the litter was considered the experimental unit. Occasionally, the data were calculated using both the litter and the fetus as the unit for comparison. Averages were calculated for each litter, then across litters. The chi square and student's "t" test were used to perform statistical analyses.

Results and Discussion

The calculations for computing the Prenatal Development Index are presented in Table 2. The first part of the Prenatal Development Index involving the vehicle control data was calculated in the following manner. A ratio was taken between the number of live fetuses and the total number of implantations. This then takes into account the number of dead, malformed or pathological fetuses. Thus, if all the fetuses were live and normal, the value for A' would be one. This was rarely seen in the laboratory and the value for A' was usually less than one. A small value for A' indicates that there were few live, normal fetuses relative to the number of implantations. The other part of the vehicle control data for consideration was the fetal weight. In these calculations, the fetal weight was taken as a ratio of itself which gave a value for B'. The fetal weight could have been compared to the fetal weight from non-treated mice to correct for any effect the vehicle might have had. However, in these experiments, it was elected to

Table 1. Chemical and common name of compounds studied

Compound common name	Compound chemical name/composition	Source	Label information reference/or lot no.	Assay (% purity)	TCDD [b] (ppm)
2,4-D	2,4-Dichlorophenoxyacetic acid	Aldrich Chem. Co.	D7072	90.0	—
2,4-D *iso*propyl ester	2,4-Dichlorophenoxyacetic acid, *iso*propyl ester	Dow Chemical Co.	072442	99.0	—
2,4-D *n*-butyl ester	2,4-Dichlorophenoxyacetic acid, *n*-butyl ester	Dow Chemical Co.	AGR 97643	98.4	—
2,4-D *iso*octyl ester	2,4-Dichlorophenoxyacetic acid, *iso*octyl esters	Dow Chemical Co.	540103	97.0	—
2,4-D PGBE	2,4-Dichlorophenoxyacetic acid, propylene glycol butyl ether esters	Dow Chemical Co.	PP1-93-1	99.9	—
2,4-D butyric acid[c]	4-(2,4-Dichlorophenoxy) butyric acid	Chipman Chem. Co.	407281	99.6	—
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid	Dow ChemicalCo.	T00206-2	99.9	<.05
2,4,5-T *n*-butyl ester	2,4,5-Trichlorophenoxyacetic acid, *n*-butyl ester	Dow Chemical Co.	PP1-93-3	99.2	<.1
2,4,5-T *iso*octyl ester	2,4,5-Trichlorophenoxyacetic acid *iso*octyl esters	Dow Chemical Co.	PP1-93-5	96.8	<.1
2,4,5-T PGBEE	2,4,5-Trichlorophenoxyacetic acid, propylene glycol butyl ether esters	Dow Chemical Co.	PP1-93-6	99.4	<.5
2,4,5-T butyric acid	4-(2,4,5-Trichlorophenoxy) butyric acid	PTSEL respository[a]	128	99.6	—
2,4,5-TCNB	2,4,5-Trichloronitrobenzene	Aldrich Chem. Co.	T5520	—	—
2,4,6-T	2,4,6-Trichlorophenoxyacetic acid	K & K Labs, Inc.	19296	—	—
Silvex	2-(2,4,5-Trichlorophenoxy) proprionic acid	Dow Chemical Co.	PP1-93-7	99.6	<.1
Agent Orange	2,4,-D n-butyl ester 45.5% 2,4,5-T n-butyl ester 48.2% inert ingredients 6.3%	Pentagon	6840-926-9095	—	0.4
3,4-D	3,4-Dichlorophenoxyacetic acid	Aldrich Chem. Co.	1200481	—	—

[a] PTSEL = Pesticide and Toxic Substances Effects Laboratory, EPA
[b] TCDD = 2,3,7,8 tetrachlorodibenzo-*p*-dioxin
[c] = Also known as 2,4-DB and Embutox

Table 2. Calculations for the Prenatal Development Index

$\frac{\text{TOTAL NO. NORMAL LIVE FETUSES—VEHICLE CONTROL}}{\text{TOTAL NO. IMPLANTATIONS—VEHICLE CONTROL}} = A'$	$A' \times B' = C$
$\frac{\text{AVG. FETAL WEIGHT—VEHICLE CONTROL}}{\text{AVG. FETAL WEIGHT—VEHICLE CONTROL}} = B'$	
$\frac{\text{TOTAL NO. NORMAL LIVE FETUSES—EXPERIMENTAL}}{\text{TOTAL NO. IMPLANTATIONS—EXPERIMENTAL}} = A$	$A \times B = E$
$\frac{\text{AVG. FETAL WEIGHT—EXPERIMENTAL}}{\text{AVG. FETAL WEIGHT—VEHICLE CONTROL}} = B$	
$\text{PRENATAL DEVELOPMENT INDEX} = \frac{E \times 100}{C}$	

consider any vehicle effects as negligible and the fetal weights observed in the vehicle control mice as normal. In order to magnify any effect on the fetuses, the values for A' and B' were multiplied to give the control value C.

The same type of calculations relative to the number of normal live fetuses and implantations was done for the experimental mice to arrive at values for A and B. For the B value, the fetal weights of the experimental mice were taken as a ratio to the vehicle control fetal weights. By using the same control fetal weight for the denominator in calculating both B and B', any effect that may have been produced by the vehicle was cancelled out. The values for A and B for the experimental animals are multiplied to give the experimental value E. The number E evaluates the overall development of the experimental animals while the number C gave the same information for the control animals. A direct comparison of these two numbers would give the information needed in any one experiment. But since it was often necessary to compare data either between different experiments or between replicates of the same experiments, it was necessary to normalize these values. This was done by taking the experimental value E as a percent of the control value C. Thus, by establishing the control value C as 100% and calculating E as a percent of C, the resultant value is the Prenatal Development Index.

Many different experiments were done with the herbicides and related compounds. The control data from these experiments are presented in Table 3. For most of the studies the vehicle was corn oil:acetone (9:1) administered by gastric intubation daily at a volume of 0.1 ml/mouse/day. The days of administration vary between experiments since part of these experiments were designed to maximize the response on palate development. The maternal weight gain and the maternal liver to body weight ratio are the two parameters used to indicate maternal toxicity. These data in Table 3 from the various individual experiments are considered normal. Each experiment had a series of control data and all statistical comparisons have been made between the experimental data and its respective control data in this table.

In addition to the maternal data, Table 3 presents the fetal information that was the basis for deriving the Prenatal Development Index. The number of implantations included the number of live fetuses, dead fetuses, and resorption sites. In general, when compound administration began after implantation, this value was not affected. All of the fetal values in Table 3 are in the normal range

Table 3. Control data for all experiments

Vehicle	Gest. days dosed	No. of litters	Maternal weight gain (g)		Maternal liver/body weight × 100		Total no. impl.	No. live normal fetuses	Avg. % fetal mort.	Avg. fetal weight (g)		$A' \times B' = C$[d]
			$\bar{X}$	SD	$\bar{X}$	SD				$\bar{X}$	SD	
None	–	5	8.39	1.85	6.12	0.17	62	54	11	0.92	0.07	.87
Oil:ac[a]	7–15	16	5.59	1.39	6.85	0.37	196	172	12	0.98	0.11	.88
Oil:ac	10–15	7	5.28	1.97	6.90	0.54	80	75	6	0.95	0.07	.94
Oil:ac	11–13	9	5.05	1.32	6.35	0.28	112	99	11	0.94	0.07	.88
Oil:ac	12–15	12	5.77	1.04	5.88	0.51	126	108	13	1.01	0.10	.86
Oil:ac 2[b]	12–15	8	3.81	1.02	6.30	0.25	103	83	21	0.96	0.05	.81
DMSO-sc[c]	12–15	13	4.73	2.34	6.29	0.43	171	152	12	1.03	0.13	.89
Corn oil	12–15	7	4.52	1.56	6.19	0.62	88	74	14	1.05	0.14	.84

[a] Corn oil/acetone (9:1) administered orally at 0.1 ml/mouse/day
[b] Corn oil/acetone (9:1) administered orally at 0.2 ml/mouse/day
[c] Dimethyl sulfoxide administered subcutaneously at 0.1 ml/mouse/day
[d] Defined in Table 2

for the CD-1 mouse. This range of variation of fetal weights was seen in my laboratory although all mice were killed on day 18 of gestation. Because of this variation it was difficult to add data from two experiments even though all other aspects of the experiments were the same. No cleft palates were seen in any of the control fetuses.

The last column in Table 3 presents the *C* value. The range of *C* values reflects both normal variation between different sets of mice and any vehicle effects.

The first set of experiments was undertaken with the herbicide 2,4,5-T. Table 4 presents the maternal data for these experiments. There was no significant difference in the maternal weight gain in any of the groups. However, the group receiving 2,4,5-T in 0.2 ml of corn oil:acetone/day gained less weight than any other group. The large standard deviation precluded this small population from being statistically significant and suggested that this group of mice was beginning to show a toxic effect. The increased amount of vehicle may have enhanced the bioavailability of 2,4,5-T. The maternal liver-to-body weight ratio was significantly increased in all groups of 2,4,5-T treated mice. This had been reported in previous studies with 2,4,5-T. In general, the magnitude of the liver response reflects the maternal exposure to the herbicide.

The fetal data are presented in Table 5. The data for the average fetal weight and the average percent cleft palates are presented in two ways since there has currently been so many papers discussing the statistical approaches to this type of data as discussed later. The first set of values for each parameter was calculated based on the total number of fetuses while the second set of values

Table 4. Maternal toxicology data from CD-1 mice after administration of 2,4,5-T

Vehicle	Dose/day m*M*/kg	Gest. days dosed	No. of litters	Maternal weight gain (g)		Maternal liver/ body weight × 100	
				$\bar{X}$	SD	$\bar{X}$	SD
Oil:ac[a]	0.45	10–15	10	5.85	2.88	7.80	1.13
Oil:ac	0.80	11–13	9	5.18	0.89	7.54[f]	1.12
Oil:ac	0.80	11–14	5	6.94	1.77	7.45	0.45
Sucrose[b]	0.80	11–14	6	7.29	1.60	7.07[f]	0.69
DMSO-po[c]	0.80	11–14	6	4.65	2.54	7.28[g]	0.62
Oil:ac	1.00	12–15	8	7.26	2.32	7.61[h]	0.84
Oil:ac/2[d]	1.00	12–15	7	1.66	3.78	7.92	0.93
DMSO-sc[e]	1.00	12–15	6	3.06	2.44	8.20[g]	1.26

[a] Corn oil/acetone (9:1) administered orally at 0.1 ml/mouse/day
[b] Solution (15%) administered orally at 0.1 ml/mouse/day
[c] Dimethyl sulfoxide administered orally at 0.1 ml/mouse/day
[d] Corn oil acetone (9:1) administered at 0.2 ml/mouse/day
[e] Dimethyl sulfoxide administered subcutaneously at 0.1 ml/mouse/day
[f] $p = 0.05$
[g] $p = 0.01$
[h] $p = 0.001$

Table 5. Prenatal evaluation of 2,4,5-T in CD-1 mice

Vehicle[a]	Dose mM/kg	Gest. days dosed	Total no. implant	No. normal live	Avg. % fetal mortality	Av. fetal weight (g)/ Total fetuses	Litter X	Litter SD	Av. % cl. palate/ Total fetuses	Litter	Prenatal Development Index
Oil:ac	0.45	10–15	107	86	17	0.89	0.89	0.07	7	6	80
Oil:ac	0.80	11–13	122	88	14	0.87	0.87	0.10	16	14	76
Oil:ac	0.80	11–14	59	21	29[b]	0.87	0.89	0.11	48	37	37
Sucrose	0.80	11–14	79	64	17	0.97	0.98	0.11	2	1	100
DMSO-	0.80	11–14	62	15	42[c]	0.95	0.95	0.22	52	49	29
Oil:ac	1.00	12–15	82	75	8	0.96	0.96	0.17	1	1	100
Oil:ac/2	1.00	12–15	94	49	41[c]	0.75	0.75	0.06	11	10	51
DMSO-sc	1.00	12–15	68	11	72[c]	0.70	0.66	0.13	48	67	11

[a] Vehicles as given in Table 4
[b] $p = 0.05$
[c] $p = 0.001$

was based on the average of the litter. In the first experiment, 2,4,5-T was administered on days 10 through 15 of gestation at a dose of 0.45 m*M*/g per day using corn oil:acetone as the vehicle. This procedure did not have any significant effect on fetal mortality or fetal weight. There was an average of 6% cleft palates produced per litter. No cleft palates were observed in any of the control fetuses. Very few randomly occurring malformations other than cleft palates were seen in these experiments and they would cancel out in computing the Prenatal Development Index. Although there was no significant increase in fetal mortality, there was a modest increase over the control value. This calculated to give a Prenatal Development Index of 80. This means that the calculated value for "E" was 80% of the control value "C." This indicated that these fetuses from the 2,4,5-T treated mice were 80% as normally developed as their respective controls. When the dose of 2,4,5-T was increased almost twice to 0.8 m*M*/g, the number of days of compound administration was reduced to prevent any possible maternal toxicity. Fetal mortality and fetal weights were not affected by this increased dose. However, more cleft palates were produced and the Prenatal Development Index dropped to 76.

The next three experiments show the variation that could be achieved depending upon whether 2,4,5-T was in solution or suspension. In the first experiment, 2,4,5-T was in solution in the corn oil:acetone mixture; in the second experiment, 2,4,5-T was in suspension in a 15% sucrose solution; and in the third experiment, 2,4,5-T was in solution in DMSO. Since these three experiments were coincident, the common control was the non-treated group. All statistical comparisons were made accordingly. All other experiments had a concurrent vehicle control as indicated in Table 1. In the experiments utilizing corn oil and DMSO solution, there was a significant increase in fetal mortality. Fetal weights were not greatly affected by any procedure. This is a good example of obtaining one toxic response, namely lethality without obtaining the other toxic response of decreased fetal weight. Cleft palates were produced in all experiments, but the response was minimal with 2,4,5-T in suspension in a sucrose solution. 2,4,5-T administered as a suspension in a sucrose solution was almost ineffective in producing cleft palates and fetal toxicity in contrast to 2,4,5-T in solution in either corn oil or DMSO. The Prenatal Development Indexes reflect the differences in magnitudes of the responses. A Prenatal Development Index of 100 as seen in the sucrose experiments is possible to achieve if the experimental fetal weights are greater than the control weights. This was the case in this experiment. This variation in response with the different vehicles suggests that the bioavailability of the 2,4,5-T differs with the various experimental procedures.

Three more experiments were undertaken to maximize the response of cleft palate production. The dose of 2,4,5-T was increased and the time period of administration was shifted to gestational days 12 through 15 to achieve full coverage of the time of palate closure especially if there were delayed closure. In the first of these experiments, the 1.0 m*M* dose of 2,4,5-T exceeded the solubility characteristics of corn oil:acetone (0.1 ml/mouse) and this dose of 2,4,5-T was almost a solid bolus. Data from this experiment should be compared with the data on the following line in which the volume of the vehicle was increased to 0.2 ml/mouse, which solubilized some of the 2,4,5-T and suspended the remainder.

The results of the two experiments are quite different. The compound in the bolus produced no significant affect on fetal mortality or fetal weight and only a few cleft palates. This same dose of 2,4,5-T in a solution/suspension state produced a marked effect on these three parameters. This was reflected in the Prenatal Development Index of 51 which was half the value of the first experiment. To put it another way, 2,4,5-T in a solution/suspension was twice as effective as 2,4,5-T in a bolus in terms of adversely affecting fetal development. In the third of this series of experiments, 2,4,5-T in a solution of DMSO was injected subcutaneously and a maximum response was obtained. The fetal mortality was very high and fetal weights were very low, and many of the surviving fetuses had cleft palates. This marked effect was demonstrated with a Prenatal Development Index of 11. This experiment can be compared to the other experiment using DMSO even though the experimental design was slightly different. In both experiments there was an increase in fetal mortality and many cleft palates were produced, In the first experiment there was no effect on fetal weight, but in the second experiment there was a marked effect on fetal weight. It is difficult with this limited information to ascribe the effect on fetal weight to the higher dose or to the fact that it was administered a day later in gestation or that the routes differed or to a combination of these. But it was obvious that with a little shifting in the experimental design, the results of cleft palate, fetal mortality, and fetotoxicity can be produced independently.

The values for fetal weight and cleft palate were calculated in two different ways. There has been some discussion in the literature as to which might be the best way (Weil 1974, Palmer 1974, Kalter 1974, Staples and Haseman 1974, and Becker 1974). This discussion was mainly whether to calculate the averages using the fetus as the unit, or to obtain averages for the litter and then average those values thus using the litter as the unit. In most cases, the values obtained were the same for both methods of calculation. In a few cases there was a small difference. In one case, the last experiment in Table 5, there was a great difference in the two values. This was often the case when there was very few live fetuses in the litters. When there were so few fetuses to average, a slight shift in a fetal weight can make a big difference.

In the calculations for the Prenatal Development Index, the number of normal live fetuses over the total number of implantations was based on the total number and not the averages for the litters. This was necessary since occasionally there were no normal live fetuses in a litter due to the high incidence of fetal mortality and malformations.

In calculating the values with fetal weight, all live fetuses were weighed, both normal and abnormal, so there was always a fetal weight value. In cases where there was 100% fetal mortality, those litters would not enter into these calculations. For calculating the Index, the litter average for the fetal weights was used. This revolved around a practical laboratory operation. It was more expedient to weigh an entire litter and arrive at an average value than to weigh the individual members of the litter. The difference in the Prenatal Development Index using both methods of calculation was negligible and was noticable only in the studies with maximum effects. In these situations the Index was so low that a slight change in the Index would not alter the interpretation of the data. The important

point is the need to be consistent in the manner of calculation in order to make comparisons between experiments.

Comparable studies were undertaken with the esters of 2,4,5-T and these data are presented in Table 6. All of these esters were administered as a solution in 0.1 ml of corn oil:acetone/mouse/day. The *n*-butyl esters did not affect the maternal weight gain. The maternal liver to body weight ratio was affected by the administration of the butyl ester at either a low dose for a long period of administration or a high dose for a short period of administration. Both the *iso*octyl and PGBE esters produced a significant increase in the maternal weight gain at the lowest dose. The higher doses did not affect this parameter. The esters were quite similar to 2,4,5-T in that they produced an increase in the liver-to-body weight ratios. It should be noted that with each of these esters, there was a very large standard deviation with the maternal weight gain at the highest dose. This large standard deviation suggests that a toxic situation was approached with some of the mice even though the averages were not statistically different from the control values. Fetal mortality was significantly increased and fetal weights decreased at the highest dose levels with each of these esters. These high doses which resulted in increased fetal mortality and low fetal weights also resulted in reduced maternal weight gains. Upon examination of the data it was apparent that the litters with the greatest mortality and lowest fetal weights had the lowest maternal weight gains.

With the low dose of *iso*octyl ester administered for a long time period, there was a significant decrease in fetal weight but it was not statistically significant. In general, when there was a decrease in fetal weight there was also a high incidence of cleft palates. This was in contrast to the results in Table 5 with 2,4,5-T in which cleft palates could be produced without an effect on fetal weight. The Prenatal Development Index in each case reflects that a low dose given for an extended period was deleterious to fetal development, and a high dose given for a short period of time was more effective in adversely affecting fetal development.

The herbicide 2,4-D and its esters were also tested orally using a solution in corn oil:acetone, and a suspension in a sucrose solution, and subcutaneously as a solution in DMSO. In the experiment using the sucrose solution there was a significant increase in maternal weight gain. This was not seen in the other experiment using the sucrose solution. The fetal weights were significantly increased in this experiment which might contribute to the maternal weight gain. In contrast, administration of 2,4-D in DMSO produced a significant decrease in the maternal weight gain which may reflect some maternal toxicity. The liver-to-body weight ratio was significantly increased by all regimens except the sucrose solution. None of the esters affected the maternal weight gain but significantly increased the liver to body weight ratios. In contrast, the esters of 2,4,5-T were more active in increasing liver weights. In general, the greatest effects of 2,4-D were produced by the low doses administered over long time periods.

In the experiment with the sucrose solution, there was an increase in fetal mortality but no cleft palates were produced. Since there were no malformations and the fetal weights were significantly increased, the resulting Prenatal Development Index was close to normal. This result is comparable to the 2,4,5-T experiment. When 2,4-D was administered at the high dose on days 12 through 15 in both corn oil and DMSO, the Prenatal Development Indexes were equiva-

Table 6. Toxicologic and prenatal evaluation of esters of 2,4,5-T, 2,4-D and esters of 2,4-D and related compounds in CD-1 mice

Compound	Vehicle[a]	Dose m*M*/kg	Gest days dosed	No. litters	Maternal weight gain (g)		Maternal liver/ body weight × 100		Tot. no. impl.	No. norm live	Av. % fetal mort.	Fetal weight (g)		Av. % cleft palates	Pre Dev Index
					$\bar{X}$	SD	$\bar{X}$	SD				$\bar{X}$	SD		
2,4,5-T esters															
n-Butyl	Oil:ac	0.45	7–15	6	7.15	1.30	8.68[c]	0.63	68	54	20	0.84	0.07	3	77
n-Butyl	Oil:ac	0.45	10–15	7	7.33	1.55	7.18	0.70	81	75	4	0.97	0.11	4	101
n-Butyl	Oil:ac	0.80	11–13	10	4.10	1.22	6.70	1.06	118	92	9	0.85[b]	0.08	15	80
n-Butyl	Oil:ac	1.00	12–15	8	5.26	3.57	8.43[d]	0.68	80	34	33[d]	0.81[c]	0.17	37	40
*Iso*octyl	Oil:ac	0.45	7–15	4	9.10[c]	1.12	8.60[c]	0.69	50	33	18	0.79[c]	0.80	20	60
*Iso*octyl	Oil:ac	0.45	10–15	7	6.98	1.87	7.34	1.08	81	70	11	0.91	0.04	1	88
*Iso*octyl	Oil:ac	0.80	11–13	9	6.26	1.22	7.54[d]	0.44	115	102	10	0.93	0.14	1	100
*Iso*octyl	Oil:ac	1.00	12–15	8	3.31	5.79	8.78[d]	0.67	88	38	33[d]	0.79[d]	0.13	37	40
PGBE[a]	Oil:ac	0.45	7–15	10	7.20[b]	1.15	7.41[c]	0.52	116	98	15	0.90	0.10	1	88
PGBE	Oil:ac	1.00	12–15	8[c]	3.08	4.17	8.57[d]	1.28	64	34	25[d]	0.91[c]	0.14	21	56
2,4-D	Oil:ac	0.56	7–15	14	6.07	1.99	7.72[c]	0.69	184	160	12	0.86	0.16	2	88
2,4-D	Sucrose	0.80	11–14	6	6.16[b]	1.51	6.31	0.69	67	53	33[b]	0.98[c]	0.06	0	98
2,4-D	Oil:ac	1.00	12–15	8	4.80	1.92	6.81[c]	0.68	82	63	21	0.79[d]	0.09	6	70
2,4-D	DMSO-sc	1.00	12–15	8	2.46[c]	2.19	6.88[c]	0.38	105	73	27[c]	0.95	0.16	5	70
2,4-D esters															
*Iso*propyl	Oil:ac	0.56	7–15	10	5.97	0.71	7.60[d]	0.62	132	119	10	0.83[b]	0.18	0	88
n-Butyl	Oil:ac	0.56	7–15	9	5.95	1.82	7.85	0.53	123	112	9	0.92	0.13	0	98
n-Butyl	Oil:ac	1.00	12–15	9	5.17	2.22	6.47[b]	0.81	94	68	16	0.86[c]	0.15	15	71
*Iso*octyl	Oil:ac	0.56	7–15	11	5.45	1.65	7.46[d]	0.44	150	132	12	0.90[b]	0.14	0	92
PGBE[a]	Oil:ac	0.56	7–15	10	5.29	2.43	7.44[b]	0.88	106	73	25	0.81[c]	0.14	8	65
PGBE	Oil:ac	1.00	12–15	7	5.99	2.56	6.61	0.74	71	47	21	0.86[c]	0.12	16	65
Related compounds															
2,4,6-T	Oil:ac	0.80	11–13	7	3.55[b]	1.29	6.62	0.34	91	83	9	0.87	0.08	0	99
3,4-D	Oil:ac	1.00	7–15	6	3.54[c]	1.04	6.65	0.42	86	71	11	1.18[b]	0.06	0	92
2,4-D Butyric	Oil:ac	0.80	11–13	8	4.70	1.14	7.02[b]	0.44	97	86	18	0.92	0.17	0	99
2,4,5-T Butyric	Oil:ac	0.80	11–13	10	5.08	1.76	6.92	0.66	120	102	12	0.89	0.09	2	92
Silvex-po	Oil:ac	1.50	12–15	7	6.75[c]	2.66	10.27[d]	0.98	107	88	11	0.83[c]	0.08	7	88
Silvex-sc	DMSO	1.50	12–15	8	6.87[c]	1.02	9.22[d]	0.55	132	96	25[c]	0.87[d]	0.09	3	66
2,4,5 TCNB	Oil:ac	1.00	12–15	7	5.86	0.82	6.35	0.55	86	72	16	1.04	0.14	0	101
Agent orange	Oil:ac	1.00	12–15	8	5.28	1.00	7.78[c]	0.96	110	101	6	0.88[c]	0.08	3	95

[a] PGBE = propylene glycol butyl ether ester
[b] $p = 0.05$
[c] $p = 0.01$
[d] $p = 0.001$
[e] Vehicles as in Table 4

lently low. This is in contrast to the 2,4,5-T experiments. When 2,4,5-T was tested with these vehicles and with this experimental design, the results and the Prenatal Development Index varied for these two vehicles. The Index was 51 for corn oil:acetone group and 11 for DMSO group. The herbicide 2,4,5-T was more effective in producing adverse effects on fetal development than 2,4-D. When 2,4-D was administered in corn oil, the major effect was on fetal weights while fetal mortality was more affected when DMSO was the vehicle. So far, these three parameters of malformations, fetal toxicity and fetal mortality appear to vary independently.

In general, the esters of 2,4-D significantly decreased fetal weight but did not affect mortality. Cleft palates were not produced with the low doses of the esters except the PGBE ester. The *n*-butyl and the PGBE ester were the only ones studied at the high dose level and they produced a comparable incidence of cleft palates. Those Prenatal Development Indexes were 71 and 65 respectively. The responses were comparable and should be contrasted to the Prenatal Development Index of the compounds at the low dose level in which the *n*-butyl ester did not seem to affect fetal development and the Index was 98. The PGBE ester at the low dose decreased fetal weights and produced cleft palates and the Index was the same as that derived from the experiments with the high dose level. Thus, the *n*-butyl ester of 2,4-D administered at a low dose for an extended period of time did not affect fetal development but a high dose for a short period of time did adversely affect fetal development. The PGBE ester produced equivalent adverse effects on fetal development whether a low dose was given for a long period or a high dose for a short time. The other esters need further testing in this regard.

A group of related compounds were selected for study and these data are presented in Table 6. The non-herbicidal compounds, 2,4,6-T and 3,4-D both significantly decreased maternal weight gain but did not affect the liver-to-body weight ratio. Since both 2,4,5-T and 2,4-D produced an increase in the liver-to-body weight ratio, it appears that this response was dependent on the placement of the chlorine atoms in the molecule. Replacing the acetic acid in the molecule with butyric acid reduces the herbicidal effectiveness of the compound. In the studies with the butyric acid derivative there was no affect on maternal weight gain and only the dichlorocompound increased the liver to body weight ratio.

Silvex, a herbicide in which the acetic acid has been replaced by proprionic acid, was administered either in corn oil:acetone orally or DMSO subcutaneously. It should be noted that the dose was 1.5 m*M* per kg per day. The dose was increased because this compound was generally less toxic. The most remarkable result was the significant increase in the maternal weight gain which was probably due to the increased liver weight. The liver weights were greatly increased as reflected in the liver-to-body weight ratios of 9 and 10. The trichloronitrobenzene compound did not affect any of these parameters. Thus, it seems that the phenoxy acid portion of the molecule was necessary for the activity. Agent Orange, one of the herbicides used as a defoliant in Viet Nam, is a 50-50 mixture of the *n*-butyl esters of 2,4-D and 2,4,5-T. It did not affect the maternal weight gain but did increase the liver-to-body weight ratio.

2,4,6-T did not affect fetal development which was reflected in an Index value of 99. 3,4-D produced an increase in the average fetal weight. Whether this was

fortuitous or real could not be determined at this time. An Index of 92 suggests that it did not greatly affect fetal development. 2,4-D butyric acid was without an effect on fetal development while the trichloronitrobenzene compound produced some cleft palates. This was in contrast to the proprionic acid derivative, Silvex. In both experiments with Silvex, there was a decrease in fetal weights and a production of cleft palates. Fetal mortality was increased when DMSO was used as the vehicle. The Prenatal Development Index indicates that a greater affect in general was produced when Silvex was administered as a DMSO solution. The trichloronitrobenzene compound was without affect on fetal development. Even though the 2,4,5 trichloro configuration in the molecule appears to be necessary for maximum fetal affect, unless the rest of the molecule has the proper configuration, no fetal affects can be produced. Agent Orange did reduce fetal weight and produce some cleft palates; however, the affects were mild, resulting in a 92 for the Prenatal Development Index.

In order to permit a direct comparison of the data from the most commonly used herbicides, the Prenatal Development Indexes of these compounds are presented in Table 7. The herbicides 2,4-D and 2,4,5-T and Silvex, when administered at high doses for short periods during gestation either subcutaneously in DMSO or orally in corn oil:acetone, adversely affected fetal development. In the experiments with 2,4-D, the vehicle and route of administration made no difference since the index was 70 in both cases. With 2,4,5-T and Silvex, there was a marked difference in the results depending on the modes of administration. In general, Silvex was less active in affecting fetal development than the other two herbicides since the dose was much greater. The maximum effect obtained with Silvex was by administration in DMSO and in this case the Prenatal Develop-

Table 7. Comparison of the Prenatal Development Index of some common herbicides

	Treat days 12–15			
		Prenatal Development Index		
Compounds	Dose m*M*/kg	DMSO - SC	Corn oil:AC-O	
2,4-D	1.00	70	70	
2,4,5-T	1.00	11	51	
SILVEX	1.50	66	88	
	Treat days 12–15		Treat days 7–15	
Compounds	Dose m*M*/kg	Index	Dose m*M*/kg	Index
2,4-D BUTYL	1.00	71	0.56	98
2,4,5-T BUTYL	1.00	40	0.45	77
AGENT ORANGE	1.00	92		

ment Index was comparable to that obtained with 2,4-D. The most active compound of the three was 2,4,5-T and the severity of the response varied with the experimental design.

Agent Orange, a 50:50 mixture of the butyl esters of 2,4-D and 2,4,5-T produced some cleft palates but in general was less active in adversely affecting fetal development than other herbicides. A 1.0 m*M* dose of Agent Orange would be equivalent to a 0.5 m*M* dose of 2,4,5-T butyl ester combined with a 0.5 m*M* dose of 2,4-D butyl ester. It appears possible that these two esters do not act by similar mechanisms since they did not produce additive results when tested as Agent Orange. In other words, the average of the Indexes of 98 and 77 of the two esters resulted in 88 which was comparable to the Index of 92 of Agent Orange. If the esters were acting by a common mechanism, it would be assumed that an Index equal to or greater than that produced by 2,4,5-T butyl ester would result.

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